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14. ABSTRACT

Collagen is the most important structural protein in the animal kingdom and is responsible for the strength and integrity of many tissues like bone, teeth, cartilage and tendons. The mechanical properties of these tissues are determined by their hierarchical structure. However, how each level contributes to the overall properties of collagenous tissues, and how individual hierarchical levels interact, remains poorly understood. Furthermore, despite extensive experimental and computational studies including both the single molecule and macro-scale

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Intermolecular slip mechanism in tropocollagen nanofibrils

We report a detailed study of the shear interaction between two tropocollagen molecules, a major mechanism that contributes to the fibril mechanical behavior. Using steered molecular dynamics simulations in explicit solvent, we model the slip of two tropocollagen molecules at varying pulling rates. We find that the adhesion strength is highly sensitive to the pulling rate, and that it converges to a value of $10.12\,\mathrm{pN\,\AA^{-1}}$ for vanishing loading rates. We find that intermolecular H-bonds play a key role in determining the resistance against slip. Our results provide quantitative details on this mechanism of load transmission inside collagen fibrils and fibers, which is crucial for the development of constitutive models of collagenous tissues at larger hierarchical levels. Such constitutive models of collagenous tissue mechanics have many applications, ranging from development of bio-inspired materials to studies in tissue engineering. By incorporating pathological collagen mutations, our studies could advance our knowledge of mechanisms underlying important collagen-related diseases like Osteogenesis Imperfecta or Ehlers–Danlos Syndrome.

Keywords: Collagen; Shear; Nanomechanics; Steered molecular dynamics; Adhesion strength; Materiomics

1. Introduction

Collagen is the primary protein responsible for the structural integrity of all vertebrates and many other organisms. In tissues such as bone, tendons, skin and cartilage, collagen is hierarchically arranged in fibrils and different super-fibrillar structures, providing resistance to tensile stress that is crucial for the physiological function of these tissues [1–3]. The structural basis for all collagenous tissues is a triple helical tropocollagen molecule, which assembles into staggered assemblies that form collagen fibrils (see Fig. 1).

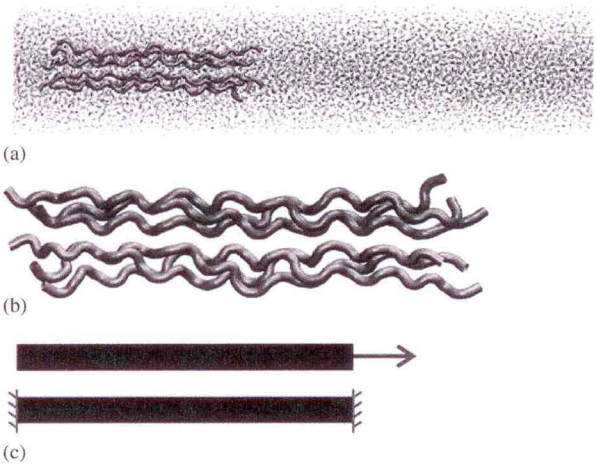

Fig. 1. Tropocollagen molecular models and loading condition. (a) depicts the molecular structure, solvated in a water box. (b) shows the details of the model geometry of the tropocollagen molecules. Both tropocollagen-like peptides considered in this study share the same structure, consisting of three identical chains made of glycine–proline–hydroxyproline triplets: $[(GPO)_{10}]_3$. (c) depicts the loading condition, subjecting the tropocollagen molecules to a shear test. The terminal ends of the lower molecule are kept fixed using position restrains, while the other molecule is pulled along its axis.

In spite of its extreme importance, the assembly, structure and the relationship between structure and mechanical properties of collagenous tissues are still not completely understood. In particular, how each level of the hierarchical structure contributes to the overall properties of collagenous tissues is poorly known. Furthermore, despite extensive experimental and computational studies both at the single molecule [4–7] and at the macro-scale level [8–11], few studies have focused on the collagen fibril level. In fact, only recently have both atomic force microscopy and microelectromechanical systems been successfully applied to assess the mechanical properties of collagen fibrils [12–14]. However, characterizing and, in turn, predicting the behavior of collagen materials requires the understanding of the mechanical properties of the substructures within the hierarchy and the interaction between the substructures. This will enable us to predict how modifications in the building blocks and their interactions influence the properties at higher levels.

Improvement in these areas could also help to significantly improve our understanding of mechanisms and treatment options of pathological conditions associated with collagenous tissues. One of the possible tools to gain more insight into the hierarchical structure of collagen fibrils and to test structural hypotheses is given by molecular simulations. With such an approach, the validity of specific structural model assumptions can be verified by agreement between experimentally measured mechanical properties and properties obtained by simulations using a mechanical model of the tissue.

Molecular dynamics (MD) simulations have already been successfully applied to study and gain insights into the nanomechanics of single tropocollagen molecules [15–19]. The goal of the present research is to use MD simulations to investigate the shear interaction between two tropocollagen molecules, a major mechanism contributing to the fibril mechanical behavior.

2. Computational procedure

The shearing mechanism of tropocollagen molecules is investigated using steered molecular dynamics (SMD) simulations. To apply mechanical load, we restrain one tropocollagen molecule while pulling the second along its main axis, as shown in Fig. 1c. This pair of tropocollagen molecules forms collagen nanofibril, representing a simplistic model of a collagen fibril without covalent crosslinks.

2.1. Molecular model implementation

We create the tropocollagen molecule models using the software THeBuScr (Triple-Helical collagen Building Script) [20, 21]. We choose the simplest model of tropocollagen, with only Gly-Pro-Hyp (GPO) triplets on each of the three chains as the reference system (Hyp and O are respectively the three letter code and single letter code for the amino acid hydroxy proline). The tropocollagen models considered here are truncated at 30 amino acids per chain in order to reduce computational costs. This leads to short length tropocollagen segments with a length of approximately 8 nm. For reasons of comparison, peptides of comparable length are considered both in computational and experimental studies [15–17, 22–24].

The initial structure for the adhesion strength calculations is obtained by using the protein structure with Protein Data Base identification 1X1K as a template. This protein structure features two collagen-like peptides juxtaposed along their principal axis. To achieve this, we superimpose two copies of the GPO peptides described above (see Fig. 1) to match the template geometry.

2.2. Molecular model equilibration

Molecular dynamics simulations are performed using the GROMACS code [25] and the GROMOS96 43a1 force field, as used in earlier studies [17–19], which also includes parameters for the hydroxyproline residue. The protein molecules are entirely solvated in a 3.3 nm × 4.7 nm × 24 nm periodic water box (to ensure a minimum distance of 0.8 nm between the proteins and the box edge; the water box is shown in Fig. 1a). Single point charge water molecules are used for the solvent, leading to a total of ≈36 500 atoms. The SETTLE (for water) and LINCS algorithms are used to constrain covalent bond lengths that involve hydrogen atoms, thus allowing a MD time step of 2 fs. Nonbonding interactions are computed using a cutoff for neighbor list at 1 nm, with a switching function between 0.8 and 0.9 nm for Van der Waals interactions, while the particle-mesh Ewald sums method is applied to describe electrostatic interactions. The preliminary system energy minimization is performed by using a steepest descent algorithm until convergence or for a maximum of 10 000 steps. The system is then equilibrated at a temperature of 310 K (37 °C) for 1200 ps of molecular dynamics. The proteins are held fixed for the first 200 ps by restraining the atomic positions, and thereafter only the first and the last C_α atoms of each chain are restrained for the following 1000 ps. The average intermolecular distance at the end of the equilibration stage is 1.2 nm.

2.3. Shear simulations

The configuration at the end of the equilibration stage is then used the as starting point for the subsequent SMD simulations. During the shear simulations the C_α atoms of one peptide are kept fixed by means of position restraint, while the center of mass of the three C-terminal C_α atoms of the second peptide (the pulled group of atoms) is linked to a spring with an elastic constant k_{spring} = 4000 kJ mol^{-1} nm^{-2}, which is moved along the direction of the molecular axis with varying velocities, from 10 to 0.1 m s^{-1} (see Fig. 1c).

All molecular dynamics simulations are carried out in an *NPT* ensemble (that is, with constant number of particles *N*, constant pressure *P*, and constant temperature *T*), with the systems coupled to a heat bath at 310 K (coupling constant of 0.1 ps and Berendsen thermostat) and to an hydrostatic bath at 1 atm (coupling constant of 0.5 ps and Berendsen barostat). The force applied to the tropocollagen molecule by the virtual spring is:

$$F(t) = k_{spring}(x_{spring}(t) - x_{pull}(t)) \quad (1)$$

where x_{spring} and x_{pull} represent the spring's and the pulled group's positions, respectively.

3. Results and discussion

We perform shear calculations (schematic see Fig. 1c) at five different pulling rates: 0.1, 0.5, 1, 5 and 10 m s^{-1}. We find that the resulting force levels are rate dependent, as the maximum force needed to slide one molecule respect to the other decreases with decreasing pulling rates, assuming values ranging from 854.6 pN to 1881 pN (see Fig. 2). Fitting a quadratic function to the force–shear rate plot leads to a simple constitutive equation to predict the shear strength $F_{max}(v)$ of a collagen nanofibril as a function of shear rate v. It is given by

$$F_{max}(v) = av^2 + bv + c \quad (2)$$

where $a = -6.6253$ pN s^2 m^2, $b = 169.19$ pN s m^{-1} and $c = 850.13$ pN. The equation can be rewritten to predict the shear strength $\tau_{shear}(v)$ (= maximum shear force per unit length of a molecule), leading to

$$\tau_{shear}(v) = \frac{1}{L_0}(av^2 + bv + c) \quad (3)$$

where the peptide length is $L_0 = 84$ Å. The quadratic extrapolation yields a maximum force at vanishing deformation rates of 850.13 pN, and an adhesion strength for vanishing loading rates of 10.12 pN $Å^{-1}$. This value in good agreement with previous studies [16, 26].

Our atomistic-level simulations provide direct insight into the molecular mechanism of shear transfer and associated rupture mechanisms. Our results show that the adhesion strength is mediated via H-bond clusters that form between neighboring tropocollagen molecules. H-bonds thereby provide the chemical basis for mechanical load transfer between tropocollagen molecules, and are thus expected to be crucial in determining the mechanical properties of collagen fibrils. The observations in the simulations suggest that the load applied to a fibril is sustained through two mechanisms: (i) molecular elongation (active at lower forces) and (ii) molecular slippage (active at larger forces) due to repeated breaking of H-bonds. This observation agrees with suggestions put forth based on earlier experimental studies [27].

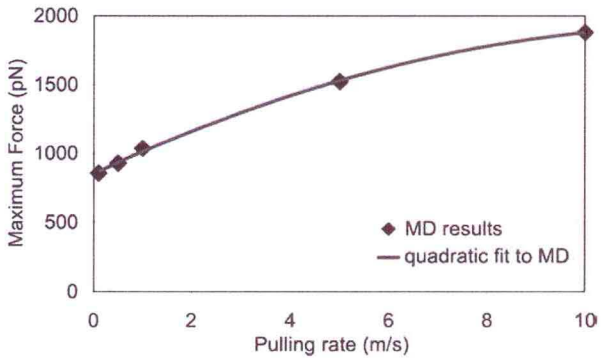

Fig. 2. Maximum force as a function of pulling rate. The maximum force needed to slide one molecule with respect to the other is found to increase with the pulling velocity. The quadratic fit allows us to determine the maximum force at vanishing rates (850.13 pN) and, through dividing by the molecular length (84 Å), the adhesion strength per unit length for quasi-static conditions (10.12 pN $Å^{-1}$). The continuous line shows a quadratic fit of the form $F_{max}(v) = av^2 + bv + c$, where $a = -6.6253$ pN s^2 m^{-2}, $b = 169.19$ pN s m^{-1} and $c = 850.13$ pN.

A more detailed analysis of the force history during the shearing process reveals that the force decreases as the adhesion area decreases. However, the decrease is not continuous, but instead features a series of force peaks. These force peaks appear to be regularly spaced. In order to explain this pattern we consider the details of the molecular interactions, which are mediated through H-bonds. Throughout the entire shearing simulation, we calculate the number of intermolecular H-bonds. Based on this analysis we find that the number of H-bonds is correlated to the force displacement history, as shown in Fig. 3. The correlation between the number of intermolecular H-bonds and the shear force suggests that the two phenomena are related, and that the force peaks are the consequence of simultaneous rupture and reformation of multiple H-bonds. This mechanism provides strong evidence for the concept that H-bonding between tropocollagen molecules is indeed a major mechanism in controlling mechanical load transfer. Figure 4 displays snapshots as the collagen nanofibrils undergo shear deformation and finally fails. It is noted that mechanisms other than H-bonding could be involved in the intermolecular interactions, such as electrostatic and hydrophobic interactions as well as intermolecular cross-links. Indeed, it has been shown that the presence of water affects the intermolecular adhesion force [28]. The analysis of these aspects is left to future work.

What is the structural basis for the observation that H-bonds form and break almost simultaneously? In order to gain insight into this question we determine the positions of H-bonds donors and acceptors involved in forming direct H-bonds, based on the three dimensional structure of the tropocollagen molecule. We pay particular attention to those molecular domains that are geometrically close enough to the opposite molecule to form H-bonds. We find that the surface profile of the tropocollagen molecule consists of crests and valleys, as visualized in Fig. 5. H-bond donors and acceptors that are capable of forming direct links with the other tropocollagen molecule are clustered in the crests of the molecular profile. This is shown in Fig. 6. This structural analysis provides a structural expla-

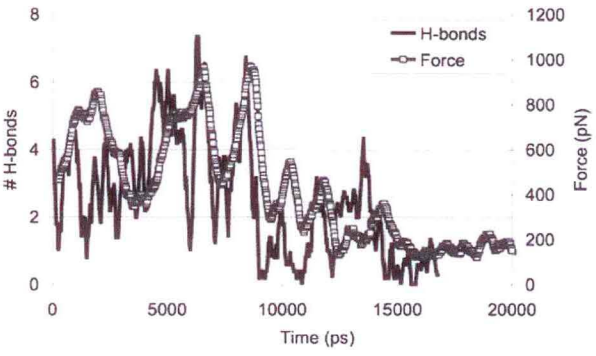

Fig. 3. Force and H-bonds number as a function of time during the shearing simulations. After reaching a peak, the force needed to slide one molecule with respect to the other decreases with time. The detailed dependence of the force includes several local maxima and minima. Further, it matches the trend of the number of intermolecular H-bonds, suggesting that the force peaks are due to simultaneous stretching and breaking of several H-bonds. The data shown correspond to a simulation with a pulling speed of 0.5 m s^{-1}. For H-bond definition, an angle donor-hydrogen-acceptor of 30° and a cut-off distance of 3.5 Å between the donor and the acceptor are considered in a geometric analysis.

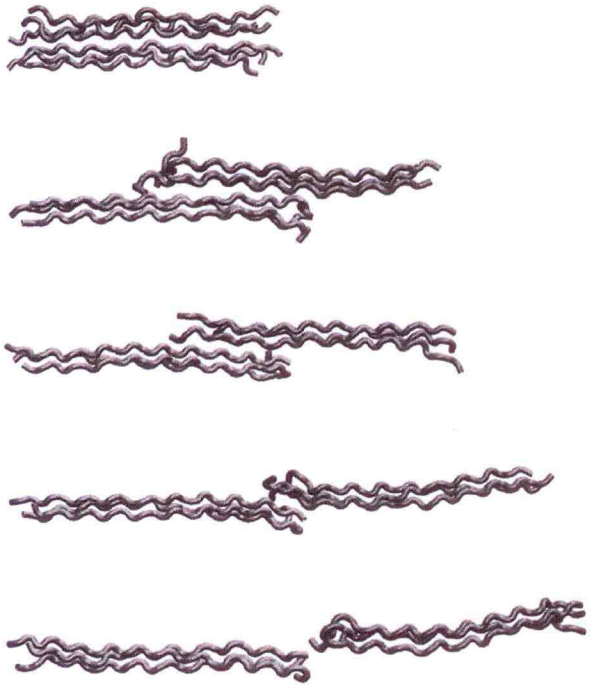

Fig. 4. Snapshots of the molecular shearing process. The images show the mechanism of shear from the initial equilibrated stage to the failure of the entire fibril.

Fig. 5. Molecular surface of tropocollagen molecule. The tropocollagen peptides present a "Lego-like" saw tooth profile due to the circumeferential rings of proline and hydroxyproline residues, which point outward with respect to the molecular axis. During the relative sliding of two tropocollagen molecules this contributes to the cyclic increase and decrease of the adhesion force as shown in Fig. 3.

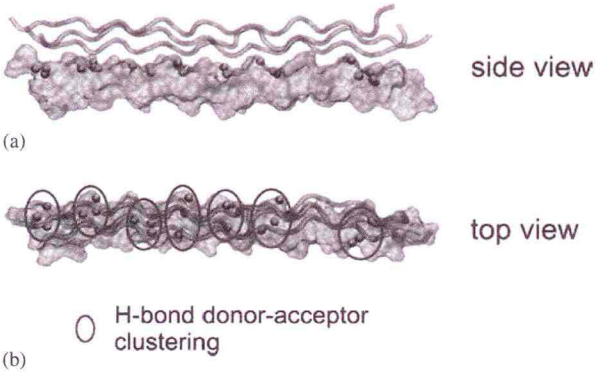

Fig. 6. (a) Side view and (b) top view of the molecular configuration at the end of the equilibration stage, showing the positions of H-bond donors and acceptors within a 3.5 Å range from the other molecule, thus able to form direct intermolecular H-bonds. The clustering of the H-bonds donors and acceptors is highlighted in (b) (a total of 7 clusters are identified, corresponding roughly to the number of peaks in the force-history shown in Fig. 3.

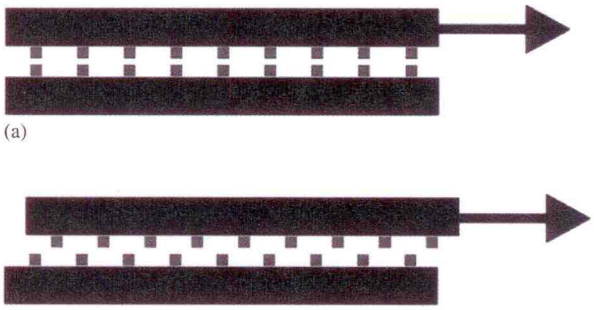

Fig. 7. Schematic model showing how the clustering of the H-bonds donors and acceptors could affect the intermolecular adhesion force profile. (a) Configurations in which many H-bonds are formed concurrently are followed by (b) configurations in which all H-bonds have been broken. The repeated occurrence of formed–broken configurations leads to the force peaks and valleys identified in Fig. 3. It is noted that this is a simplistic representation, since in the actual molecular geometry the clusters are not precisely equispaced and the molecules are somewhat flexible. Thus it is expected that H-bonds do not form and break at exactly the same time.

nation for the proposed mechanism. The particular "Lego-like" structure of the tropocollagen molecule leads to concurrent breaking and formation of intermolecular H-bonds. Therefore, during molecular sliding we expect stages in which all clusters of H-bond donors and acceptors of the two molecules are aligned, and thus a large number of bonds is observed (see Fig. 7a). Upon further shear displacement, these H-bonds break almost simultaneously, leading to a force peak at the point of rupture (see Fig. 7b). The repetition of these two stages leads to the saw tooth-like behavior of the adhesion force. Moreover, as the adhesion surface decreases, the maximum number of possible intermolecular H-bonds decreases, which is reflected in a reduction in force levels as larger parts of the molecules have been sheared.

4. Conclusions

This work elucidates the details of molecular interactions in collagenous tissues, and explains how the mechanical load is transferred in collagen fibrils by linking the molecular structure with deformation mechanisms. Since collagen fibrils are the major building blocks of many load-bearing tissues such as bone, tendon, cartilage and skin, the findings of this research are significant to advance our general understanding of the chemomechanical basis of load bearing protein materials in biology. The force levels of approximately 800 pN correspond roughly to strength measurements obtained in beta-sheet based H-bonded structures [29, 30]. These beta-sheet protein based H-bonded structures display similar structural features to the present case in collagen nanofibrils, where multiple H-bonds are arranged in parallel and loaded in parallel. This comparison provides further support for the significance of H-bonds in controlling the intermolecular shear strength. In this scenario, the effect of solvent on the mechanics of collagen fibrils could perhaps be explained by changes in the intermolecular H-bonding due to the effect of presence or lack of water molecules. Further analysis could be carried out in future work.

After identifying the genetic code of several species, an outstanding grand challenge in the life sciences is now the understanding of the multi-scale behavior of hierarchical protein assemblies. The advancement of this field is crucial for studies of biological systems, disease diagnosis and treatment, as well as the design of novel biomaterials. Studies as reported in this paper contribute to these efforts by providing structure–property links of hierarchical protein materials by quantifying the chemomechanical basis of fundamental material concepts.

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